Agricultural Analytical Chemistry
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DETERMINATION OF FENARIMOL[1/] IN MEAT, MILK, AND EGGS

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PRINCIPLE

Procedures are described for the determination of fenarimol in meat, milk, and eggs. The tissue samples are finely ground, the egg samples are blended, and milk is assayed as received. The sample extracts are purified by liquid-liquid partitioning and florisil column chromatography. The fenarimol content is measured by gas chromatography using an electron capture detector.

REAGENTS

1. Methanol, reagent grade
2. Acetonitrile, reagent grade, redistilled
3. Hexane, reagent grade
4. Methylene chloride, reagent grade, redistilled
5. 1-chlorobutane, reagent grade, redistilled, purified over florisil column; 350 ml of florisil 100/200 mesh
6. Toluene, reagent grade, redistilled
7. Methanol:acetonitrile 50:50 (V/V)
8. 1-chlorobutane:hexane 50:50 (V/V)
9. Methylene chloride:methanol 99.5:0.5 (V/V)
10. Sodium chloride, 5%/o aqueous solution
11. Sodium sulfate, anhydrous, methanol washed
12. Florisil, 100/200 mesh, 8%/o water deactivated
13. Fenarimol standard solution

EQUIPMENT

1. Sample grinding and blending equipment
2. Chromatographic columns 250 mm x 14 mm i.d. equipped with Teflon stopcock
3. Folded filter paper - Schleicher and Schuell No. 588, 15 cm
4. Rotary vacuum evaporator - Rinco (or equivalent)
5 Gas chromatograph equipped with electron capture detector

PROCEDURE

A. Preparation of Standard and Standard Curve

1. Prepare a 50 mcg/ml standard solution - accurately weigh 10 mg of fenarimol reference standard. Transfer to a 200-ml volumetric flask and dilute to mark with toluene.

2. Prepare standard solutions in toluene over the range of 0.01 mcg/ml to 0.3 mcg/ml for the standard curve.

3. Prepare a standard solution in 1-chlorobutane for fortifying recovery samples of fat and skin tissue at 0.2 mcg/ml level.

1/ α-(2-chlorophenyl)-α-(4-chlorophenyl-5-pyrimidinemethanol), coded EL-222.

4. Prepare a 50-mcg/ml standard solution in methanol - accurately weigh 10 mg of fenarimol reference standard. Transfer to a 200-ml volumetric flask and dilute to mark with methanol. Make appropriate dilutions in methanol to obtain standard solutions for fortifying recovery samples at the following concentration:

   a. Lean, liver, kidney, and eggs - 0.2 mcg/ml
   b. Milk - 0.04 mcg/ml

B. Sample Preparation

1. Lean, Liver, Kidney, Fat/Skin

   a. The frozen tissue should be finely ground and thoroughly mixed to provide a homogeneous sample.

2. Eggs

   a. The egg white and yolks should be mixed by any suitable means to provide a homogeneous sample.

3. Whole Milk

   a. The whole milk should be thoroughly mixed to provide a homogeneous sample.

C. Extraction of Lean, Liver, and Kidney

1. Weigh a representative 20.0-g sample into a half-pint Mason jar.

2. Prepare recovery samples at the 0.01 ppm level by adding 0.2 mcg of fenarimol to a 20.0-g control sample.

3. Add 86 ml of methanol:acetonitrile 50:50, (moisture content of tissue is considered so final volume will be 100 ml).

4. Blend on blender for 2 minutes using a high speed setting.

5. Filter the extract through the folded filter paper, S and S, No. 588.

6. Transfer a 20-ml (20%) aliquot of the filtered extract to a 250-ml separatory funnel.

7. Add 40 ml of 5% aqueous sodium chloride solution to the separatory funnel.

8. Extract with two 25-ml portions of methylene chloride and combine extracts in a 125-ml boiling flask. To prevent water from being present in extract, filter through sodium sulfate granules before collecting in boiling flask. Wash the sodium sulfate with methylene chloride and collect wash in boiling flask.

9. Evaporate the methylene chloride using a rotary vacuum evaporator and a 40-45°C water bath.

10. Proceed to Section G (Purification).

D. Extraction of Fat/Skin

1. Weigh a representative 20.0-g sample into a 250-ml beaker.

2. Prepare recovery samples at the 0.01 ppm level by adding 0.2 mcg of fenarimol (1-chlorobutane) to a 20.0-g control sample.

3. Place samples on steam bath until fat is melted, approximately 80°C. Add 40 ml of hexane:1-chlorobutane 50:50 and allow to come to boil. Transfer the sample to a florisil column, as prepared in Section G (Purification), by filtering the sample through a glass funnel containing a glass wool plug. Rinse beaker with two 10-ml volumes of hexane:1-chlorobutane 50:50 and add to column.

4. Proceed to Step 3 in Section G (Purification).

NOTE: Continue through purification steps; if column is allowed to cool, the fat will solidify on the column.

E. Extraction of Milk

1. Weigh a representative 40-g sample (40 ml) and transfer to a 250-ml separatory funnel.

2. Prepare recovery samples at the 0.001 ppm level by adding 0.04 mcg of fenarimol to a 40-g control sample.

3. Add 40 ml of acetonitrile to the separatory funnel and mix thoroughly.

4. Add 40 ml of hexane to the separatory funnel, shake, and allow thorough separation (approximately 20 minutes).

5. Drain acetonitrile (lower phase) to a second 250-ml separatory funnel.

6. Wash the hexane with an additional 20 ml of acetonitrile, allow to separate, and combine acetonitrile portions in the second 250-ml separatory funnel.

7. Add 60 ml of methylene chloride to the combined acetonitrile extracts and partition acetonitrile into the methylene chloride.

8. Drain methylene chloride (lower phase) through sodium sulfate granules into a 250-ml boiling flask.

9. Wash sodium sulfate granules with methylene chloride and collect wash in boiling flask.

10. Evaporate methylene chloride using a rotary vacuum evaporator and 40-45°C water bath.

11. Proceed to Section G (Purification).

F. Extraction of Eggs

1. Weigh a 20.0-g representative sample into a half-pint Mason jar.

2. Prepare recovery samples at the 0.01 ppm level by adding 0.2 mcg of fenarimol to a 20.0-g control sample.

3. Add 84 ml of methanol to sample (moisture content of sample is considered so final volume will be 100 ml).

4. Blend on blender for two minutes using a high speed setting.

5. Filter the extract through folded filter paper, S and S, No. 588.

6. Transfer a 40-ml (40%) aliquot of the filtered extract to a 250-ml separatory funnel.

7. Add 80 ml of 5% aqueous sodium chloride solution to the separatory funnel.

8. Extract with two 30-ml portions of methylene chloride and combine extracts in a 125-ml boiling flask. To prevent water from being present in extract filter through sodium sulfate granules before collecting in boiling flask. Wash the sodium sulfate with methylene chloride and collect wash in boiling flask.

9. Evaporate the methylene chloride using a rotary vacuum evaporator and a 40-45°C water bath.

10. Proceed to Section G (Purification).

G. Purification

1. Prepare a florisil column for each sample as follows:

a. Place a pledget of glass wool into the bottom of a 14 mm i.d. glass chromatographic column. Add 15 ml of hexane:1-chlorobutane 50:50, and tamp the glass wool with a stirring rod to eliminate air.

b. Add 10 ml ± 0.5 ml of standardized florisil through a funnel. Add 5-10 ml of hexane:1-chlorobutane 50:50, and stir with a rod. Rinse the sides of the column with additional solvent.

NOTE: See Section H (Standardization of Florisil) for preparation of absorbant. The florisil must be added to the columns in a reproducible manner; therefore, assuring a consistent elution pattern for all samples within a set.

c. After the florisil settles, add about 1.5 cm of anhydrous sodium sulfate, layering it carefully to avoid disturbance of the florisil surface. Rinse sides of the column with additional solvent.

2. Quantitatively transfer the residue to the column using two 10-ml volumes of hexane:1-chlorobutane 50:50. Allow each addition of solvent to pass through the absorbant at a flow rate of 3-5 ml per minute.

3. Wash the column with 50 ml of hexane:1-chlorobutane 50:50.

4. Wash the column with 20 ml of methylene chloride. Discard the eluates through this point.

5. Elute the residue with 40 ml of methylene chloride:methanol 99.5:0.5 and collect in a 125 ml boiling flask.

   NOTE: The above describes column parameters taken in the author's laboratory. There is lot-to-lot variation in florisil activity, and each lot should be standardized as described in Section H.

6. Evaporate the column eluant by a rotary vacuum evaporator.

7. Dissolve the residue in the boiling flask in the appropriate volume of toluene, mix thoroughly, and proceed with gas chromatographic measurement.

   Lean, liver, kidney, milk, and eggs - 1.0 ml
   Fat/Skin - 5.0 ml

H. Standardization of Florisil

1. The florisil as received must be deactivated prior to use with the addition of distilled water. Determine the loss on drying of the absorbant as received (normally 1-2 percent). Add sufficient distilled water to an appropriate amount of florisil to give a total moisture content of 8.0 percent. After addition of the water, stir gently with a rod to break up any lumps. Tumble the florisil for 1 hour in a closed container and allow to stand for 2 hours prior to use.

2. Determination of column parameters.

   a. Prepare a florisil column as described in Section G, Step 1, under "Procedure".

   b. A control and recovery for each tissue type will need to be passed over the florisil columns to determne the elution pattern with tissue extract present.

   c. Wash the column as described in Section G, Steps 2 and 3 under "Procedure" and discard.

   d. Elute the column with 20 ml of methylene chloride and collect in a 125 ml boiling flask.

e. Elute the column with 50 ml of methylene chloride:methanol 99.5:0.5. Collect the first 20 ml volume and then in 10-ml volume increments.

f. Evaporate all of the fractions collected and bring up in the appropriate volume of toluene for gas chromatographic measurement.

g. Quantitate the column check against standard solutions of equal concentration and determine what fractions must be collected to establish $\geq$ 90% recovery.

I. Gas Chromatography

Gas Chromatograph:	Hewlett-Packard Model 5713A, equipped with a Ni-63 electron capture detector or an equivalent GC system.
Column:	120 cm x 2 mm i.d., glass, packed with 3% OV-17 on Chromosorb W-HP 80/100 mesh.
Alternate Column:	120 cm x 2 mm i.d., glass, packed with 2% OV-17 on Chromosorb W-HP 80/100 mesh.
Temperatures:	Column Oven - 260°C Injection Port - 300°C Detector - 300°C
Electrometer	Set the attenuation to provide 50% full scale deflection from the injection of 0.2 ng of fenarimol standard.
Carrier Gas:	50-60 ml/min 90:10 argon:methane

With the above conditions, the retention time of fenarimol is approximately 8 minutes.

NOTE: These instrument conditions are intended as guidelines. Actual conditions may vary.

J. Measurement

1. Inject standard curve, control, standard recoveries, and experimental samples into the gas chromatograph using the instrument conditions previously described.

2. An injection volume of 5.0 µl is recommended; however, this may be adjusted to suit the needs of the analyst. The manual injection technique may be any of those described in the U.S. Health, Education, and Welfare Pesticide Analytical Manual, Volume I, Section 302.31.

3. Using the measured peak heights and the standard curve data, determine the concentration of fenarimol, in mcg/ml, in the control, standard recoveries, and experimental samples. If the peak height of any solution is not within the standard curve range, make appropriate dilutions and reinject solutions.

K. Calculations

1. Recoveries

$$\text{Percent Recovery} = \frac{\text{mcg/ml (from Std Curve)} \times SV \times AF \times 100}{\text{mcg Fortified}}$$

Where:
SV = final sample volume
AF = aliquot factor

2. Parts-per-million

$$\text{ppm} = \frac{\text{mcg/ml} \times SV \times AF \times DF \times 100}{\text{sample weight (g)} \times \text{percent recovery}}$$

Where:
DF = dilution factor (1.0 unless sample is diluted)

DISCUSSION

A. Validation of the assay method was performed with control and recovery tissue samples on three different days. The recovery levels used for validation were the detection limits desired for each tissue.

Summary of Daily Validation Runs

Tissue	PPM Fortified	N	Percent Recovery[1/] Mean ± SD
Lean	0.01	4	114.5 3.3
	0.01	4	107.3 9.9
Liver	0.01	4	80.8 7.2
	0.01	4	112.8 9.2
	0.01	4	115.0 7.1
Kidney	0.01	4	95.8 7.2
	0.01	4	113.8 2.5
	0.01	4	114.8 6.2
Fat/Skin	0.01	4	87.8 3.8
	0.01	4	88.5 3.3
	0.01	4	76.3 4.8
Milk	0.01	4	96.0 2.4
	0.001	4	99.5 8.4
	0.001	4	81.0 5.9
	0.001	4	82.3 5.7
Eggs	0.001	4	117.0 3.6
	0.01	4	99.5 7.9
	0.01	4	92.8 5.2
	0.01	4	102.8 11.8

1/ Notebook Reference: 1P5

Summary Data - Method Validation

Tissue	PPM Fortified	N	Percent Recovery1/ Mean	± SD
Lean	0.01	12	100.8	16.5
Liver	0.01	12	107.8	11.5
Kidney	0.01	12	105.4	13.7
Fat/Skin	0.01	12	86.9	9.1
Milk	0.001	16	94.9	16.4
Eggs	0.01	12	98.3	9.0

B. The presence of tissue extract appears to enhance the GC response of fenarimol and should be considered when using the standard curve data. The response can be normalized by injecting 2-3 control tissue samples before injecting the standard curve.

An alternate column clean-up procedure to be considered if interferences are encountered is a non-deactivated alumina. The elution guidelines for a 13 ml wet packed (methylene chloride) alumina column are as follows:

Two 5-ml volumes of methylene chloride to transfer residue.

Wash column with 20 ml of 99:1 methylene chloride:methanol and discard. Elute residue with 30 ml of 99:1 methylene chloride:methanol. Collect and evaporate for residue assay.

These parameters are guidelines and column checks will be needed to determine the elution pattern for different lots of alumina.

The whole milk used in this method was fresh, non-homogenized milk, and was maintained under refrigeration. Poor phase separation during liquid-liquid partitioning was encountered with older milk; therefore, the milk to be assayed by this procedure should not be more than 3 days old.

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